Mercury Methylation and Demethylation Studies at Southern Indian Lake, Manitoba: 1981-1983

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March 1987

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1490

Fisheries and Oceans / Pêches et Océans

Canada

87-02094/

Canadian Technical Report of Fisheries and Aquatic Sciences

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by

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This is the 3rd Technical Report

from the Central and Arctic Region, Winnipeg

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[1] Agassiz North Associates

1214 Chevrier Blvd.

Winnipeg, MB R3T 1Y3

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Cat. no. Fs 97-6/1490E ISSN 0706-6457

Correct citation for this publication is:

Ramlal, P.S., C. Anema, A. Furutani, R.E. Hecky, and J.W.M. Rudd. 1987. Mercury methylation and demethylation studies at Southern Indian Lake, Manitoba: 1981-1983. Can. Tech. Rep. Fish. Aquat. Sci. 1490: v + 35 p.

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From 1981 to 1983, studies were made on the processes of mercury methylation and demethylation at the Southern Indian Lake (SIL) reservoir. Specific rates of mercury methylation measured in the lake sediments during a survey of flooded lakes was generally related to the total mercury measured in fish muscle. Limnocorral studies demonstrated how an actively methylating microbial community becomes established after major environmental perturbations. These populations could become established under conditions where nutrients and mercury were readily available. In the limnocorrals used for more than one field season, the bacteria began to produce methyl mercury as soon as more mercury was added to the limnocorrals. A number of different materials, such as clay-mineral sediment and moss, were tested to determine their effect on mercury methylation. Moss in the short term could inhibit methylation, possibly by absorbing the added mercury. SIL bank material (clay-rich) bound less mercury than clay sediment from a mercury contaminated lake in Ontario, Wabigoon Lake, and much of the bound mercury could later be released.

Key words: mercury; methyl mercury; methylation; demethylation; reservoirs (water); limnocorrals.

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De 1981 à 1983, on a mené des études sur les processus de méthylation et de déméthylation du mercure dans le lac Southern Indian. Les taux spécifiques de méthylation du mercure tels que quantifiés dans les sédiments lacustres au cours d'un relevé des lacs inondés étaient généralement en relation avec le mercure total présent dans les muscles de poisson. À l'aide d'études en limnocorrals, on a montré comment une communauté microbienne en état de méthylation active s'établit après d'importantes perturbations environnementales. Ces populations peuvent s'établir dans des conditions où les bioéléments et le mercure sont facilement disponibles. Dans les limnocorrals utilisés pendant plus d'une saison de travaux sur le terrain, les bactéries ont produit du mercure méthylé dès l'apport de mercure. On a mis à l'essai différentes matières dont de la mousse et des sédiments argile-minéral afin de déterminer leur incidence sur la méthylation du mercure. À court terme, la mousse peut inhiber la méthylation par absorption possible due mercure ajouté. Les matières composant les rives du lac (riches en argile) ont retenu moins de mercure que les sédiments argileux d'un lac ontarien contaminé (lac Wabigoon); la plus grande partie du mercure lié peut être libérée ultérieurement.

Mots-clés: mercure; mercure méthylé; méthylation; déméthylation; réservoirs (de l'eau); limnocorrals.

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Mots-clés: mercure; mercure méthylé; méthylation; déméthylation; réservoirs (de l'eau); limnocorrals.

INTRODUCTION

In 1976 Southern Indian Lake (SIL), in northern Manitoba, was impounded as part of the diversion of the Churchill River for hydroelectric development (Newbury et al. 1984). Fish muscle mercury levels increased in lakes flooded by the diversion and the observed increase of fish mercury levels was due to enhanced bacterial methylation of naturally occuring mercury in the flooded soils (Bodaly and Hecky 1979; Bodaly et al. 1984a).

Inorganic mercury and compounds containing mercury can be rapidly transformed by microbial action in aquatic environments. Methylated mercury is the most toxic of the compounds produced by these transformations (Brosset 1981). Environmental methyl mercury concentrations are regulated by the concurrent processes of production and degradation (e.g. Brosset 1981; Lexmond et al. 1976). Methyl mercury is biologically produced both by microorganisms which metabolize the mercuric (Hg^{2+}) ion to detoxify their environment (Pan-Hou and Imura 1982) and as a result of incorrect synthesis of organic molecules such as amino acids (Wood et al. 1972). Other bacteria then eliminate methyl mercury by converting it to methane and elemental mercury (Hg^{0}) (Tonomura and Kanzaki 1969; Tonomura et al. 1972). When inorganic mercury enters an aquatic ecosystem much of it is adsorbed on to organic and inorganic particles in the water (Benes and Havlik 1979; Rudd et al. 1980b). These organic particles (mostly phytoplankton and biological debris) can subsequently undergo decomposition, freeing previously bound inorganic mercury for biological methylation; sorption/desorption equilibria of mercury determine its distribution between dissolved and inorganic particle phases (Santschi et al. 1986). Mercury must be available as the dissolved mercuric ion for biological methylation to occur (DeSimone et al. 1973).

Methyl mercury is transferred through the food chain via a number of routes. It may adsorb onto, or be absorbed by, phytoplankton (Windom and Kendall 1979). It can also enter fish through the gills (Olson et al. 1973). Armstrong and Hamilton (1973) found that mercury concentrations of organisms in a mercury contaminated system was related to food selection. They found that omnivorous organisms, detritus feeders, and bottom dwelling invertebrates had considerably higher mercury levels than was found in either herbivorous organisms or those feeding on zooplankton. Little is known of the chemical form of mercury in invertebrates except that the percentage of total mercury methylated is quite low (Huckabee et al. 1979). Methyl mercury can also be produced by the intestinal contents of fish and bioaccumulation from the intestine can occur (Rudd et al. 1980a).

In 1981, the following studies on the microbiological transformations of mercury at SIL began. They are described under three headings:

1: Limnocorrals
Sediment was collected from limnocorrals subjected to various treatments to find out how quickly an actively methylating population of bacteria could be established, and what conditions were conducive to enhanced mercury methylation.

2: Survey Studies
Specific rates of methylation and demethylation of mercury were measured in sediment and water samples collected from flooded shorelines and offshore "old lake" sediment in and around SIL.

3: Additional Studies
a. The capacity of natural materials such as lake sediment and lake shore soils to fix or adsorb mercury was tested by examining the effect of these materials on mercury methylation.

b. Bodaly et al. (1984) found that levels of mercury in the upper organic soil horizon were much higher than inorganic subsoil layers. We have conducted an experiment to find out how erosion of this upper organic layer by flooding would affect the availability of mercury for methylation.

1: LIMNOCORRALS

METHODS AND MATERIALS

Site description

Beginning in 1981, limnocorrals were set up in a small bay off of Wupaw Bay (previously known as Long Bay) in SIL (Fig. 1.1; Hecky et al. 1987). Detailed explanation of the limnocorral experiments is available in Hecky et al. (1987). The treatments added to the limnocorrals are listed in Table 1.1. At various times during July and August of 1981, 1982, and 1983, water and sediment were collected from selected limnocorrals and the specific rates of mercury methylation and demethylation determined.

Sample collection and manipulation

Until August 23, 1981, sediment was collected using a modified Ekman dredge (Burton and Flannagan 1973). Flocculent material at the sediment water interface (0-2 cm) was siphoned with a hand vacuum pump into 500 mL PVC bottles. Water samples were obtained 30 cm above the undisturbed sediment surface. Limnocorral sediments of August 23, 1981, and all samples until August 16, 1982, were taken with a submersible pump and collected in a 20 L carboy. The suspended material was allowed to settle overnight, the supernatant was decanted and the settled 'floc' resuspended in 500 mL of lake water.

After August 16, 1982, samples were collected from each site by a submersible electric pump the intake of which was dragged over the sediment surface and the fluid pumped into 2 L or 20 L carboys. The sediment was treated as described above before methylation measurements were done.

In 1983, the samples were collected using the same apparatus described in 1982, but the surface sediment was siphoned into 2 L PVC bottles and the supernatant was not decanted.

Radioisotope addition and sample incubation

In the laboratory, the sediment suspension was mixed and 100 mL was measured into each of three acid washed 125 mL glass reagent bottles, one as a control and two as replicate tests. When demethylation was included (in 1983) an additional three subsamples were taken. The microbial activity of the sediment controls was stopped with 1 mL of 4N HCl prior to isotope addition. Sediments collected from anoxic sites were incubated with nitrogen in the head space of the incubation vessels to maintain anaerobic conditions. All other samples were incubated with room air in the headspace. Methylation measurements of water was done using the above procedure, with 100 mL of water.

Specific rate of mercury methylation: Methylation samples were injected with ^{203}Hg as $HgCl_2$ at an approximate dosage of 74 kBq·2 µg Hg^{-1}·g dry $sediment^{-1}$. The samples were shaken for 1 min after isotope addition then incubated at in situ temperatures for 12 h. Following incubation, 1 mL of 4N HCl was added to each sample and the alkylated ^{203}Hg was extracted using an organic solvent extraction technique (Furutani and Rudd 1980). The ^{203}Hg activity of the samples was determined with a liquid scintillation counter (Packard 3033).

Specific rate of demethylation of methyl mercury: In 1983, the rate of microbial breakdown of methyl mercury, or demethylation, was determined by adding ^{14}C-methylmercuric iodide to a set of samples separate from those used for the methylation studies. The isotope was added at a dose of 14.8 kBq·0.2 µg Hg (as $CH_3HgI)^{-1}$·g dry $sediment^{-1}$. The samples were shaken for 1 min after isotope addition and incubated for 12 h at the in situ temperatures. After incubation, the samples were acidified and the volatile ^{14}C and products of demethylation were stripped from the samples (Ramlal et al. 1986). The ^{14}C activity of the samples was determined with a liquid scintillation counter (Packard 3033).

Calculation of specific rate of biological mercury methylation and degradation: The following equations were used to determine the specific rate of microbial mercury methylation (M) after the radioactive counts had been quench corrected. Methyl mercury degradation (D) is calculated using the same equations.

1. Total µg Hg methylated =

$$\frac{(dpm_{sample} - dpm_{blank}) \cdot \text{scale factor}}{\text{added dpm} \cdot \mu g^{-1} \text{ Hg}}$$

2. ng Hg methylated·g dry $sediment^{-1} \cdot h^{-1}$ =

$$\frac{\text{Total µg Hg methylated} \cdot (1000 \text{ ng} \cdot \mu g^{-1})}{\text{dry weight (g)} \cdot \text{incubation time (h)}}$$

3. % of added Hg methylated·g dry $sediment^{-1} \cdot h^{-1}$ =

$$\frac{[\text{ng Hg methylated} \cdot \text{g dry sediment}^{-1} \cdot h^{-1}] \cdot 100}{\text{added ng Hg}}$$

Microbial activity: In 1982, microbial metabolic activity was measured by monitoring the oxygen consumption of the unconcentrated sediment/water mixtures using Winkler titrations (Stainton et al. 1977).

In 1983, microbial activity of the sediments was monitored by measuring the rate of production of carbon dioxide. Replicate samples of sediment were added to test tubes of a known volume, with either room air or helium (for sediments which had no oxygen in overlying water) in the head space. After incubation at the in situ temperatures for 12 h the sediment was acidified with HCl and the gas in the head space was collected for CO_2 determination by gas chromatography. Carbon dioxide was measured at the beginning and end of the incubation period. Measurement of methane production was attempted during one series of methylation/demethylation measurements, but methane could not be detected using a thermal conductivity gas chromatograph.

Chemical analyses: Subsamples of the sediment collected were taken for total suspended solids, carbon and nitrogen analyses (Stainton et al. 1977). The pH of the sediment/water suspension was measured prior to sediment incubation and dry weights of material in suspension were determined. The results of the chemical analyses of the sediment are listed in the appendix.

RESULTS AND DISCUSSION

The results of some of the mercury in fish muscle are included in this section. These data are more fully described in Hecky et al. (1987) and are included here for comparative purposes (Figs. 1.2A-B).

Specific mercury methylation rates were measured on two dates in the limnocorrals in 1981 (Table 1.2). Mercury methylation was undetectable in the water column, was barely detectable in the phosphorus and nitrogen (P and N) limnocorral, but could be measured in the moss limnocorral (0.086 % methylated·g dry $sediment^{-1} \cdot h^{-1}$). The rate measured in the P and N limnocorral was close to those measured in sediment collected outside of the limnocorrals (Bay samples in Table 1.2). Mercury methylation was undetectable in both the high clay and control limnocorrals. Yellow perch collected from the moss enclosure had the highest measured total Hg concentrations in muscle tissue (Fig. 1.2A).

In 1982, the first measured methylation rates (July 8 in Table 1.3; Fig. 1.3) were higher in both the one year old control (0.008% methylated·g dry $sediment^{-1} \cdot h^{-1}$) and the enclosure containing the previous year's moss treatment (Table 1.1). Low methylation rates

were measured in the limnocorral with bank clay addition on all of the dates it was sampled except August 23, but it still had the lowest rate measured of all the limnocorrals sampled on that date. These results are consistent with the field and laboratory studies (Section 2, Table 2.1 - South Bay and Table 2.2; Section 3, Table 3.3), in which no methylation was detected in bank clay or in clay-rich sediment. The sediment of the double moss addition had increasing specific methylation rates during the sampling period, and had the highest measured rate (0.132 % added Hg methylated·g dry sediment^{-1}·h^{-1}) of all the limnocorrals.

There was no apparent relationship between the total Hg in fish muscle (Fig. 1.3A) and the mean specific mercury methylation rates (Fig. 1.3B). The fish of the double moss addition, the bank clay addition and the one year old moss limnocorral had high mercury levels, while mean methylation rates were high in moss limnocorrals (0.032-0.054% methylated·g dry sediment^{-1}·h^{-1}) but low in the bank clay limnocorral (0.004% methylated·g dry sediment^{-1}·h^{-1}).

In 1983, specific mercury methylation rates of sediment were consistently higher in re-used limnocorrals, regardless of the treatment (Tables 1.5A-B). Degradation of methyl mercury occurred in substantial amount in all limnocorrals regardless of age or treatment. A ratio of the specific methylation rate to the specific demethylation rate (M/D) was calculated (Table 1.5B). There did not seem to be any seasonal trends of M/D in the limnocorral sediment. The mean M/D was high in all re-used limnocorrals and low in the new limnocorrals which was primarily due to the differences in the mean methylation rates. Total Hg was highest in the fish of the control limnocorral (Fig. 1.4A), although uptake of ^{203}Hg was not exceptional (Hecky et al. 1987).

The limnocorral experiments show how bacterial populations can be altered when they are exposed to environmental perturbations such as additions of mercury. When limnocorrals were used for more than one year in the same site (Table 1.4) an actively methylating population of bacteria was already established. In all the limnocorrals used more than once, the initial methylation measurements the following year were higher than in new limnocorrals (Table 1.4). In 1983, the mean M/D ratio for the season for all reused limnocorrals was at least three times higher than in new limnocorrals regardless of the treatments they were given. In the control limnocorral which had received a substantial dose of stable mercury with each radioactive spike for two years, the mean specific methylation rate was 0.069% added Hg methylated·g dry sediment^{-1}·h^{-1} and the fish mercury levels were the highest recorded for the three years of the limnocorral experiment (Fig. 1.4A and B). It seems likely that the sediment of this limnocorral had a high available mercury concentration after two mercury additions, and also a well established population of mercury methylating bacteria.

Table 1.1. Treatments used in the enclosures at SIL during 1981 to 1983. Each year, approximately 2 mg Hg was added to each limnocorral with radioisotope spike with the exception of limnocorral 2 in 1982. This increased the background mercury concentration in water by 5 to 10.

Limnocorral number	1981	1982	1983
1	control (n)	6 year-old moss undisturbed (n)	7 year-old moss (1) (flooded) disturbed
2	moss (n)	window screen (n)	spruce boughs (2)
3	P and N (n)	1x moss (n)	1x moss (n)
4	low clay (n)	2x moss (n)	control (6)
5	control (n)	1 year old control (1)	1982 clay addition (1)
6	high clay (n)	new control (n)	Two year old moss (8)
7	control (n)	bank clay (n)	1x moss (n)
8	bay	1 year old moss (2)	1x moss and lake clay addition (n)

(n) denotes new limnocorral; (1) denotes limnocorral number of previous year. In 1981, limnocorral number 8 was not a limnocorral, but the water sampled outside of the limncorrals.

Table 1.2. ^{203}Hg-methyl mercury production, SIL 1981 (as measured by % added mercury methylated·g dry sediment^{-1}·h^{-1}). Bay refer to samples taken near but outside limnocorrals.

Limnocorral	July 20	Aug. 3
moss	0.000 (w)	0.086
P & N		0.001
control		0.000
high clay	0.000 (w)	0.000
bay	0.002	0.001
bay	0.000 (w)	

(w) water samples

Table 1.3. ^{203}Hg-methyl mercury production at SIL 1982, limnocorrals (as measured by % of added mercury methylated•g dry sediment^{-1}•h^{-1}).

Limnocorral	July 8	July 21	Aug. 3	Aug. 23
6 yr moss	0.001	0.002	0.050	0.024
1x moss	0.002	0.001	0.023	0.020
2 x moss	0.005	0.005	0.072	0.132
1 yr control	0.008	0.007	0.000	0.012
control	0.003	0.002	0.001	0.015
bank clay	0.001	0.000	0.001	0.012
1 yr moss	0.012	0.004	0.002	0.109

Table 1.4. Comparison of early July (initial) mercury methylation rates for limnocorrals used more than one year (as measured by % added mercury methylated·g dry sediment^{-1}·h^{-1}).

Limnocorral	Years	Methylation Rate	
		Year One	Year Two
6-7 yr moss	1982-83	0.001	0.025
control	1982-83	0.003	0.049
clay	1982-83	0.001	0.025
moss	1982-83	0.012	0.082

Table 1.5A: Specific rates of biological methylation and demethylation of mercury by sediments collected from limnocorrals at SIL, 1983 (as measured by % of added mercury methylated or demethylated·g dry sediment^{-1}·h^{-1}).

Limnocorral	July 10, 1983		July 25, 1983		August 7, 1983	
	D	M	D	M	D	M
7 yr moss disturbed	0.099	0.025	0.153	0.065	0.228	0.032
spruce	0.041	0.039	0.230	0.069	0.306	0.057
1x moss*	0.241	0.006	0.141	0.006	0.339	0.001
control	0.159	0.049	0.180	0.128	0.310	0.029
1982 bank clay	0.259	0.025	0.372	0.086	0.206	0.049
2 yr moss	0.148	0.082	0.209	0.059	0.188	0.028
1x moss*	0.113	0.001	0.352	0.006	0.291	0.007
1x moss & lake clay*	0.065	0.001	0.174	0.022	0.286	0.018

* These limnocorrals were new in 1983 (see Table 1.1).

Table 1.5B: Methylation/demethylation ratios (M/D) and average ratios of limnocorral sediment studies of 1983.

Limnocorral	July 10	July 25	August 7	Mean for all dates
7 yr moss disturbed	0.254	0.422	0.139	0.272
spruce	0.930	0.300	0.187	0.472
1x moss*	0.024	0.043	0.003	0.023
control	0.308	0.709	0.093	0.370
1982 bank clay	0.095	0.231	0.236	0.187
2 yr moss	0.552	0.284	0.146	0.327
1x moss*	0.011	0.016	0.025	0.017
1x moss & lake clay*	0.012	0.126	0.060	0.066

* These limnocorrals were new in 1983 (see Table 1.1).

2: SURVEY SAMPLES

METHODS AND MATERIALS

In 1981, sediment was sampled from South Bay on SIL, an area of severe bank erosion and high sedimentation (Hecky and McCullough 1984; Newbury and McCullough 1984). Sediment collected from Granville Lake, an unflooded lake upstream on the Churchill River from SIL, was used as control sediment. Sediment was obtained from Issett Lake, a small lake off the Churchill diversion which had extensive flooding and low flushing. Sediment was also collected from Methyl Bay, a relatively isolated bay on SIL. Methyl Bay is relatively shallow with slumping shorelines caused by melting permafrost. The bathymetry is unrecorded but depths >8 m occur. Sediment from this bay was taken from sites which had been inundated when the lake was impounded in 1976 as well as from sites on the original lake bed.

In 1982, in addition to sediment from Methyl Bay, Granville and Issett Lakes, samples were taken from East and West Mynarski, and West Notigi as well as Areas 4 and 5 in SIL (Fig. 1.1; Newbury et al. 1984). Unflooded E. Mynarski Lake was a control site, Areas 4 and 5 of SIL and West Notigi Lake were flooded (Bodaly et al. 1984b) but Area 4 had the least areal extent of flooding of any of surveyed sites i.e. lowest area flooded as a ratio of lake area after flooding (Newbury et al. 1984).

In 1983 survey samples were collected only from Methyl Bay.

Sample collection and all of the analyses were done as described in Section 1.

RESULTS AND DISCUSSION

In 1981, methylation was measured at three sites on two different dates (Table 2.1). The highest rates were measured in sediment collected from the flooded Issett Lake. Methylation was close to zero in sediments collected from both flooded South Bay and unflooded Granville Lake. Comparison of fish muscle mercury levels and methylation measured during the 1981 mercury survey were too limited to allow conclusions to be reached and the data is not included. Methylation was measured at only three sites and there were no complete collections of yellow perch or spottail shiners for each of these three.

Five sites were studied at Methyl Bay during 1982 (Table 2.2) where methylation was only detectable in sites which had been inundated by flooding. In the clay-rich offshore sediments, at greater depths than inshore sites but similar temperatures, there was virtually no methylation. There was a strong seasonal trend at the inundated sites with maximum methylation rates occurring in early to mid-August.

Mercury methylation rates measured on sediment collected during the August 17-18, 1982 survey (Table 2.3), show that the sediment from Issett, as in the previous year, had high rates (0.312% added $Hg \cdot g^{-1} \cdot h^{-1}$) relative to controls (i.e. Granville in 1981, Granville and East Mynarski in 1982; 0-0.015% added $Hg \cdot g^{-1} \cdot h^{-1}$), W. Mynarski sediment had a similar rate of 0.261% added $Hg \cdot g^{-1} \cdot h^{-1}$. The level of mercury methylation in W. Notigi 2.25% added $Hg \cdot g^{-1} \cdot h^{-1}$ was 10 times higher than that measured at these other inundated sites.

Sediment methylation rates and total mercury in fish muscle (Bodaly et al.1987; Figs. 2.1A and B), followed similar trends. Methylation was highest in W. Notigi sediment which had the highest mercury concentration in fish muscle. Fish mercury concentration and sediment methylation rates were also comparable in W. Mynarski and Area 5, SIL samples. Of the sites which exhibited the lowest methylation rate (unflooded control sites E. Mynarski and Granville, and flooded site in Area 4 in SIL) only fish collected from Area 4 had relatively high mercury levels. It is possible that the methylating bacteria at this site were sampled during a period of decline, or that the site sampled for methylation measurement was not representative of the habitat frequented by the fish.

In 1983, methylation was almost undetectable in the offshore clay rich sites of Methyl Bay (Table 2.4), flooded inshore sites had methylation rates ranging from 0.020-0.267% added $Hg \cdot g^{-1} \cdot h^{-1}$. A comparison of the M/D of the flooded and unflooded areas within the bay show significant differences. The M/D ratios of the flooded areas were as high as 0.824. It would appear that the flooding of Methyl Bay caused a shift in the M/D ratio so that the methylating population created a higher rate of net methyl mercury production than that in the unflooded sites.

Table 2.1. ^{203}Hg-methyl mercury production, SIL 1981 (as measured by % added mercury methylated·g dry sediment^{-1}·h^{-1} or % added mercury methylated·L^{-1}·h^{-1}).

Site	July 25	Aug. 18
Granville	0.000	0.000
South Bay	0.000	0.000
Issett	0.035	0.055
Granville (w)	0.000	0.000
South Bay (w)	0.000	0.000
Issett (w)	0.000	0.000

(w) water samples

Table 2.2. ^{203}Hg-methyl mercury production; Methyl Bay, SIL 1982 (as measured by % of added mercury methylated•g dry sediment^{-1}•h^{-1}).

Site	Depth (m)	July 6	July 12	July 19	Aug. 2	Aug. 16
clay	5.0	0.000	0.000	0.000	0.000	0.000
clay	5.0		0.000			
clay	5.0	0.000	0.000	0.000	0.000	0.001
flooded	2.5	0.000	0.005	0.008	0.077	0.093
flooded	3.0	0.001	0.000	0.007	0.126	0.021

Table 2.3. Hg-methyl mercury production of 1982 SIL surveys (as measured by by % of added mercury methylated·g dry sediment^{-1}·h^{-1}).

Site	Aug. 17
Mynarski E.	0.015
Mynarski W.	0.261
Notigi W.	2.251
Area 5, SIL	0.057
Area 4, SIL	0.011
Granville	0.004
Issett	0.312

Table 2.4. Specific rates of biological methylation and demethylation of mercury and the ratio of these processes (M/D) by sediments collected from three stations at Methyl Bay, SIL 1983 (as measured by % added mercury methylated or demethylated·g dry sediment^{-1}·h^{-1}).

Station	Depth (m)	July 19, 1983			July 19, 1983		
		D	M	M/D	D	M	M/D
clay	5.2	0.023	0.003	0.110	0.067	0.001	0.009
flooded	2.6	0.205	0.149	0.730	0.267	0.220	0.824
flooded	1.7	0.134	0.020	0.147	0.184	0.025	0.137

3: ADDITIONAL STUDIES

METHODS AND MATERIALS

Samples

Materials from the shoreline and backshores of SIL were used in the following laboratory experiments. These materials included clay sediment, clay soils, humic soils, cores from a Sphagnum moss bed and surface lake water (Table 3.1). Special care was used with the material required for the erosion experiment to ensure they were undisturbed cores or large intact pieces (e.g. clay sediment) from which cores were taken. Sediment was also collected from Wabigoon Lake in Ontario. This lake has been mercury contaminated and the sediment was used as a comparison to the sediments collected from SIL.

Inhibition of mercury methylation by various materials

Various materials were tested for their ability to inhibit methylation (Table 3.1). Distilled water was used as a control i.e. sample with no ability to adsorb mercury. Acid killed blanks (no microbial methylation occurring) contained 50 mL of distilled water and 1 mL of 4N HCl. Each of the materials was added to 125 mL glass reagent bottles and autoclaved for 20 minutes at 121°C. Fify mL of well mixed sediment from Lake 114 (Experimental Lakes Area) was added to the reagent bottles and served as both an inoculum of mercury methylating bacteria and their required nutrients. The sediment from Lake 114 is not contaminated by mercury and has been previously used as a source of mercury methylating bacteria (Furutani and Rudd 1980). The sediment consisted mainly of flocculent organic material. The amount of Lake 114 sediment used was more than enough required to overwhelm any differences in the nutrient composition between the various materials tested.

Samples to be incubated anaerobically were flushed with nitrogen for 5 min. During flushing the Lake 114 sediment and $^{203}HgCl_2$ was added to each bottle. Aerobically incubated samples had room air in the headspace of the incubation vessels. The aerobic samples were incubated with, and without agitation (150 rpm) during incubation. Samples were incubated for 24 hours.

Moss basket experiment

On July 25, 1982 an experiment testing the effectiveness of moss binding and/or scavenging mercury from the water began. This experiment was done in Wupaw Bay and Wupaw Bay bog at SIL, lasting from July 25, 1982 until August 26, 1983. Bundles of moss were suspended in the water column of each site to find out whether moss would pick up mercury from the water, and if so, whether some of this mercury would be converted to methyl mercury and sequestered in the moss. At various times (Table 3.2) subsamples were taken from the moss bundles at each site and frozen for storage and subsequent analysis of total and methyl mercury.

Total mercury was measured by atomic absorption and methyl mercury was estimated using a gas chromatograph equipped with an electon capture detector (Furutani and Rudd 1980; Uthe et al. 1972). To ensure that the methyl mercury was not overestimated, silver sulphate removal of the extracted methyl mercury was also done. The samples were then re-injected into the gas chromatograph (with an electron capture detector) and the peak height of material with the same retention time as methyl mercury was subtracted from the original measurement.

Comparison of distribution of ^{203}Hg between SIL bank material/water and Wabigoon Lake/sediment water

Decreasing amounts of SIL clay-rich bank material and Wabigoon Lake sediment were dispensed into 125 mL Erlenmeyer flasks. Distilled water (10 mL) was added to each of the flasks to final as concentration water:sediment of 2:1, 6:1, 25:1, and 50:1 for the SIL bank material and 11:1, 24:1, 32:1, and 90:1 for the Wabigoon Lake sediment suspensions. Radioactive mercuric chloride was added to each of the flasks (7500 dpm/flask) which were shaken for 2 min. Subsamples were removed, centrifuged at 6000 G for 30 min, and 2 mL of the supernatant was counted in a liquid scintillation counter.

The sediment pellets from the centrifuge tubes were resuspended in water at the same concentrations and left to stand for one week. Subsamples were again centrifuged, the supernatant counted and the % of the previously adsorbed Hg that was released after one week was calculated. The distribution ratio, K_d, (Nyffeler et al. 1984) was calculated for the ^{203}Hg:

$$K_d = \frac{C_s}{C_w}$$

where C_s is the concentration of ^{203}Hg in sediment ($dpm \cdot g^{-1}$) and C_w is the concentration of ^{203}Hg in water ($dpm \cdot mL^{-1}$).

The effect of soil perturbation by erosion on mercury methylation

Mercury methylation rates were measured on a variety of materials using 20-30 g wet weight (5 g dry weight) for sediments and soils, or 50 mL of lake water. Methylation was detectable only in the clay sediment sample (Table 3.3). Therefore, the "erosion" experiment was conducted with these sediment samples, and included humic and clay soil samples for comparison. Six cores were taken from a large intact piece of clay sediment. The cores, 3 cm in diameter and 5-6 cm deep were transferred to plexiglass jars of the same diameter. Four mL of sterile, distilled water was added to each of the soil samples to rehydrate them. Duplicate or triplicate cores of each type of sample were mixed with a glass rod to simulate erosion which would destroy the bacterial micro-environments. A total

of 1 mL of $^{203}HgCl_2$ in 100 µL aliquots was injected into 10 different sites within each core. A 100 µL Hamilton syringe fitted with a fine needle was used for the injections into both the mixed and intact cores. One mL of 4 N hydrochloric acid was added to the $^{203}HgCl_2$ when the blank cores were injected. Following the 24 hour incubation the alkylated ^{203}Hg was extracted from the entire core (Furutani and Rudd 1980).

RESULTS AND DISCUSSION

Inhibition of mercury methylation of natural material

The results of this experiment (Table 3.1) have been interpreted with the assumption that high methylation rates are indicative of high dissolved (bio-available) mercury concentrations. This is a valid assumption since it has been demonstrated that mercury methylation is directly related to mercury concentration over several orders of magnitude 0.04-10 µg $Hg \cdot g^{-1}$ sediment (Rudd et al. 1983). Distilled water with Lake 114 sediment should have the most bio-available mercury (as $^{203}HgCl_2$) because it had the highest methylation rate. The methylation rates and the corresponding bio-available mercury levels are expressed as a percentage of the rate found in the control. The comparable "% unavailable mercury" is the difference between the "% bio-available mercury" and "100% bio-available mercury" (Table 3.1).

There was virtually no mercury methylation detected in samples which contained room air in the headspace of the incubation vessel and which were shaken during incubation. In the samples containing room air, but not shaken during incubation, the methylation rate was much lower than the control (Table 3.1). These results indicated that atmospheric oxygen would inhibit mercury methylation in these samples. The unshaken, aerobic sample still methylated at a reduced rate because without agitation anaerobic micro-environments were probably present in the bottom of the sample during incubation.

Moss basket experiment

Total mercury and methyl mercury analyses were made of each of the moss-peat subsamples. The results (Table 3.2) do not show any evidence of significant methyl mercury accumulation or loss by the moss-peat material on a dry weight basis from the beginning to the end of the experiment.

Comparison of distribution of ^{203}Hg between SIL bank material/water and Wabigoon Lake sediment/water

The results of addition of ^{203}Hg to SIL bank material and Wabigoon Lake sediment (Table 3.4) show there are considerable differences between the two materials. The SIL bank material was clay-rich, and the Wabigoon Lake sediment had more sand than clay. The SIL bank material had considerably lower K_d than the Wabigoon Lake sediment, that is, there was more ^{203}Hg in the supernatant. After standing for one week, more ^{203}Hg had desorbed from the SIL material and although the K_d values were higher, they were still less than that of the Wabigoon material. It is unlikely that equilibrium was established after 32 min, but enough ^{203}Hg had been adsorbed to determine the K_d one week later.

The effect of soil perturbation by erosion on mercury methylation

The highest methylation rates measured in the erosion experiment were found in clay mineral sediment cores; low rates were found in the bank soils. When sediment cores were mixed, the methylation rates of both the clay sediment and bank soil increased three-fold (Table 3.3). Mercury methylation was not detected in either mixed or unmixed cores of the clay soil.

The total and methyl mercury concentrations of the three cores were measured (Table 3.3) and the methyl mercury results agree with the methylation results. The sediment sample had both the highest methylation rate and the most methyl mercury (Table 3.3). Both the humic soil and the sediment clay had 120 ng total $Hg \cdot g$ dry $sediment^{-1}$, yet methyl mercury was undetectable in the humic soil.

Table 3.1. Effect of various materials on the bio-available mercury, SIL 1981.

Autoclaved Material	Incubation Headspace Shaken	Gas	MeHg (dpm/ sample)	Bio-availability Hg relative to distilled water	% Bound
control 50 mL dist. water	no	N_2	5638	100	0
clay 1.5g dry wt	no	N_2	3882	69	31
SIL unfilt. water 50 mL	no	N_2	3485	62	38
Sphagnum moss 1.5g dry wt	no	N_2	1075	19	81
poplar forest soil 1.5 g dry weight	no	N_2	3082	53	47
blank 50 mL acidified d water	no	N_2	27		
50 mL d water	no	air	428		
50 mL d water	yes	air	28		

* 50 mL ELA (L114) sediment (approx. 1.5 g dry weight) were added to all samples as bacterial inoculum and nutrient source.

Table 3.2. Methyl mercury and total mercury concentration of moss samples suspended in Wupaw Bay and Wupaw Bay bog, SIL.

Site	Date	Total mercury ($\mu g \cdot g^{-1}$)	Methyl mercury ($ng \cdot g^{-1}$)
Initial samples	July 15, 1982	0.08	7
		0.09	3
		0.07	4
		0.06	6
Lake	August 10, 1982	0.08	9
		0.08	<1
	October 18, 1982	0.09	<1
		0.11	<1
		0.07	<1
	August 26, 1983	0.06	3
		0.07	6
Bog	August 10, 1982	0.12	3
		0.09	8
	August 26, 1983	0.08	3
		0.08	<1

Table 3.3. Description of samples, water content, total mercury and methyl mercury concentration (per gram dry weight) and ^{203}Hg methyl mercury production in samples used for erosion experiments, SIL 1981.

Sample type	Description	% Water	Total Hg $ng \cdot g^{-1}$	MeHg $ng \cdot g^{-1}$	Sample Cond.	Methylation (dpm/sample)
Clay sediment	-fine roots and fibers throughout the core	82	120	1.8	unmixed	41
						47
					mixed	145
	-dark brown to black with grey clay sections					112
						171
Humic soil	-roots and fibers throughout the core	71	120	UD	unmixed	1
						3
	-organic texture				mixed	5
	-dark brown					11
Clay soil	-no roots or fibers	25	30	UD	unmixed	0
	-light brown, grey clay					0
					mixed	0

UD - undectectable-less than 0.3 $ng \cdot g^{-1}$ dry weight.

Table 3.4. Comparison of SIL bank material and Wabigoon Lake sediment ^{203}Hg binding and distribution ratios (K_d).

Lake	Dil'n*	dmp•mL^{-1}	dpm•g^{-1}	K_d	% bound ^{203}Hg
SIL (32 min)	2:1	34.0	1502	44.2	97.5
	6:1	39.2	4296	109.6	95.6
	25:1	304.0	11115	36.6	59.5
	50:1	272.4	23759	87.2	63.7
SIL (1 week)	13:1	26.4	9361	345.6	96.8
	25:1	65.7	9506	144.7	82.7
	50:1	79.4	19808	249.5	80.1
Wabigoon (32 min)	11:1	37.1	7160	193.0	94.4
	24:1	60.7	14811	243.9	90.9
	31:1	45.3	19460	429.6	93.2
	91:1	96.2	51986	540.4	85.5
Wabigoon (1 week)	24:1	33.8	11244	332.7	94.4
	31:1	29.6	14828	500.9	95.2
	91:1	38.2	38121	997.9	93.3

* Dilutions of the SIL bank material and Wabigoon Lake sediment were made using 10 mL of distilled water and decreasing amounts of sediment.

GENERAL DISCUSSION

The radiochemical methods we used (Furutani and Rudd 1980; Ramlal et al. 1986) measure the specific rates of methylation and demethylation. The radioactive mercury, both $^{203}Hg^{2+}$ and $^{14}CH_3Hg^+$, was added to samples in sufficient quantities to overwhelm the concentrations of these mercury species which might be present in the samples and are not conventional radiotracer methods. These methods are, however, more sensitive than those methods which use stable (cold) mercury to estimate both mercury methylation and demethylation. For detectable results the methods using stable mercury require long incubation periods and the addition of mercury at concentrations far exceeding background concentrations (and well above concentrations used in our assays; Billen et al. 1974; Spangler et al. 1973) of even the most contaminated sites (Armstrong and Hamilton 1973; Jackson and Woychuk 1980). The long incubation times and high mercury concentrations may cause the microbial populations to change from their natural state. In contrast, it is unlikely that the microbial population changed during the short incubation times required for measurable results by radioisotope assays (Kelly and Chynoweth 1980). Although the mercury concentrations used in our studies are greater than natural levels at SIL (Bodaly et al. 1984a), the amounts were not large enough to affect the microbial activity of the sediment (Furutani and Rudd 1980; Rudd et al. 1983; Ramlal et al. 1986). Therefore, the specific rates of methylation and demethylation are realistic; but, to convert them to absolute rates, the in situ concentrations of substrates, i.e. Hg^{2+} and CH_3Hg^+ would have to be known. Consequently, we use the M/D ratio to illustrate the relative balance of these rates among different sites and over time. Although M/D ratio were always less than 1.00 (e.g. Tables 1.5B and 2.4), this does not mean that absolute in situ demethylation rates exceed in situ methylation rates. The availability of substrate will also determine whether methyl mercury is accumulating or decreasing. The M/D ratio does show whether the organisms are capable of favouring either the methylation or demethylation reaction.

The mercury methylation and demethylation studies at SIL demonstrate the succession of methylating microflora which occurs after an area is flooded. Microorganisms in terrestrial environments often have limited access to water, nutrients and mercury, among other substances, which can be metabolized. In the upper organic soil layers of the shoreline around SIL, total mercury levels were much higher than in inorganic subsoil layers (Table 3.3; Bodaly et al. 1984a). If this mercury were methylated by soil microorganisms, the methyl mercury might either be released to the atmosphere (Friberg and Vostal 1972; Johnson and Braman 1974) or have a limited area of dispersal. No methyl mercury was detected in the soils used in the erosion experiments (Table 3.3) while small amounts were measured in moss-peat material used in the moss basket experiment (Table 3.2).

After an area of land is flooded, the terrestrial microhabitats of bacteria are either disrupted or completely destroyed, which will allow bacteria freer access to a wider variety of substrates. In soil, especially dry soil, microorganisms can persist in 'resting stages' until the habitat is more conducive to their growth (Atlas and Bartha 1981). The presence of water will often stimulate these resting stages, increasing the numbers of viable microorganisms (Atlas and Bartha 1981). An aquatic environment also makes substrates (e.g. nutrients) more accessible through hydration and diffusion. As the flooded terrestrial flora (trees, moss, etc.) undergo decomposition the amount of available bacterial substrate also increases. When these substrates are flooded, the microbial decomposition of this material can be quite rapid, releasing further nutrients.

In the comparison of SIL bank material and Wabigoon Lake sediment (Table 3.4) it was shown that the SIL material bound less mercury, and the mercury that was bound by SIL material was more easily released. Therefore, the addition of clay-material from the SIL banks to the lake would not have the same ameliorating effect as was found with the Wabigoon Lake sediment (Rudd and Turner 1983). They found that addition of Wabigoon Lake sediment to enclosures (limnocorrals) made ^{203}Hg less available for mercury methylation and bioaccumulation. In contrast Hecky et al. (1987) found that the addition of SIL bank material, increased mercury uptake by fish. This would mean that as long as the erosion of the banks continues, the bacteria will have available organic material, therefore, methyl mercury production can continue.

The results of the short term laboratory experiments using Sphagnum moss and ^{203}mercury (Table 3.1) indicate that moss can effectively reduce mercury methylation, while in this experiment mineral clays and lake water have a similar but much lower capacity to reduce methylation. The reduction in methylation presumably indicated that added ^{203}Hg is made unavailable to the microbes because of complexing or binding reactions with the added materials. However, in the moss basket experiment (Table 3.2) there was no detectable increase in either the total mercury or methyl mercury concentration over the duration of the experiment. This lack of evidence for significant binding of mercury is not surprising since the concentration of total mercury measured in the water of the SIL and Notigi reservoirs were less than the limit of detection (i.e. <5 ng $Hg \cdot L^{-1}$ in Bodaly et al. 1984a) which is less than that added in the laboratory experiments. Apparently, in the short term the moss can inhibit methylation possibly by sequestering dissolved mercury if it is at high concentrations, and it may not be a source of mercury. The inhibition of methylation observed experimentally (Table 3.1) may be of short duration because, as the moss is decomposed and used as a nutrient source by the microbiota, bound mercury can be released and may be methylated. The moss basket experiment would not detect such an effect because weight loss of the moss material over time was unknown.

Methyl mercury can be produced as an incorrect synthesis of the amino acid methionine (Landner 1971); and therefore, the amount of

methyl mercury produced should increase as the microbial activity increases (Jernelov 1972). Pan-Hou et al. (1980) have noted that increased sensitivity to mercury by a vitamin B_{12} auxotroph (Clostidium cochlearium T-2C) was related to the loss of ability to methylate mercury. It is unlikely that there is a feedback mechanism controlling the production of methyl mercury since methyl mercury is not utilized by bacteria. Rather methyl mercury concentration will likely be controlled by the balance of methylating and demethylating bacteria and their respective microbial activity.

The limnocorral experiments showed how bacterial populations can be altered over longer time periods (months to years) by addition of mercury compared to that found in short term laboratory studies (days). All limnocorrals received a radioisotope spike of rather low specific activity. Consequently, they all experienced a pulse of mercuric ion (Hecky et al. 1987). Methylating activity was low in new controls; but when limnocorrals were used for more than one year in the same site (Table 1.4) an actively methylating population of bacteria was already established. In all the limnocorrals used more than once, the initial methylation measurements the following year were higher than in comparable new limnocorrals. In 1983, the mean M/D ratio for the season for all reused limnocorrals was at least three times higher than in new limnocorrals regardless of the treatments they were given. Even in the reused control limnocorral the methylation rate was high and the fish mercury levels were the highest recorded for the three years of the limnocorral experiment (Fig. 1.4). After two mercury additions, this limnocorral had high available mercury concentrations and a well established population of bacteria capable of producing methyl mercury.

The short term laboratory studies and longer term limnocorrals showed how the populations of mercury methylators can become established, sometimes quite rapidly. The speed of their establishment was treatment dependent, i.e. moss-peat limnocorrals had actively methylating populations within two months while the controls required two mercury spikes and at least one year (Hecky et al. 1987). The 1983 studies in Methyl Bay may be indicative of how the lake sediment bacterial population had reacted to several years of flooding. In the offshore, clay-rich sites which had always been under water, the M/D ratio was as low as in Granville Lake which was not flooded (Fig. 2.1B). However, in the flooded sites the M/D was considerably higher (Table 2.4). The bacterial flora was conditioned to produce methyl mercury at the flooded sites seven years after flooding. The microhabitat erosion experiments show that while both the clay sediment and the clay soil contained the same amount of total mercury, it was only the clay sediment, with an actively methylating population of bacteria, which had detectable methyl mercury in the sediment (Table 3.4). If the clay soil was inundated for a longer period, the bacterial population might methylate as fast or faster than the clay sediment.

The results of the first three years of mercury methylation studies show how the net methylation of mercury at SIL might have evolved after impoundment. The older flooded sites sampled (e.g. Methyl Bay) appear to have well established microbial populations which are capable of both methylation and demethylation. Over flooded terrain the balance of these two processes had shifted in favour of net methylation compared to offshore sediments present before impoundment. The two-year limnocorrals studies show how a population of methylating bacteria can be rapidly selected if treatments supply substrate which favours rapid growth of bacteria, e.g. new moss treatments in 1981 and 1982, or slowly if only Hg^{2+} is added, e.g. in reused controls. With rapid growth the selection for methylating microorganisms can occur quickly because of the greater number of generations produced in a short time period, but the population will probably fluctuate until the conditions of the habitat are at a more constant state. Demethylation of methyl mercury seems to be regulated by factors other than the nutrient supply, since it did not follow any definable trend. The process is known to be plasmid mediated (Pan-Hou et al. 1980) and the mercuric reductase-plasmid bearing populations may not be fully established in the limnocorrals, or, the populations may be controlled by other factors in addition to methyl mercury concentrations.

Because the limnocorrals were not microbiologically in a constant state, the methylation rates measured did not always reflect the total mercury levels in the fish of these enclosures (Figs. 1.2-1.4). However, in the mercury survey of 1982 the concentration of mercury in fish muscle followed the same trends as the rate of sediment mercury methylation at the sites which had been flooded for six years (Fig. 2.1).

This research does illustrate that it is necessary to have paired methylation and demethylation to determine how the microbial population is responding to changes. Low M/D ratios would indicate that the probability of net mercury methylation was low (unless Hg^{2+} concentrations were very high) and methyl mercury would probably not be a problem. This study also shows that the amount of organic material available to the bacteria will regulate the amount of methyl mercury produced. Present research is directed towards comparison of unflooded and flooded sites in a number of lakes, similar to measurements made in Methyl Bay, so that comparison of pre-and post-impoundment sediments can be made.

ACKNOWLEDGMENTS

We thank the many people at the SIL fisheries camp for their help and cooperation in collecting field samples. F.A.J. Armstrong, R.A. Bodaly, G.J. Brunskill, P. Campbell, M. Capel, R.H. Hesslein, S.G. Lawrence, and K. Marshall, considerably improved the manuscript with their suggestions. Total mercury analyses were done by R. Hunt and sediment chemistry was done by B. Hauser. We also thank the Canadian Department of Fisheries and Oceans, and the

Canada-Manitoba Mercury Agreement for funding this research. Aspects of this study were conducted by Agassiz North Associates under DSS contract OSF83-0004.

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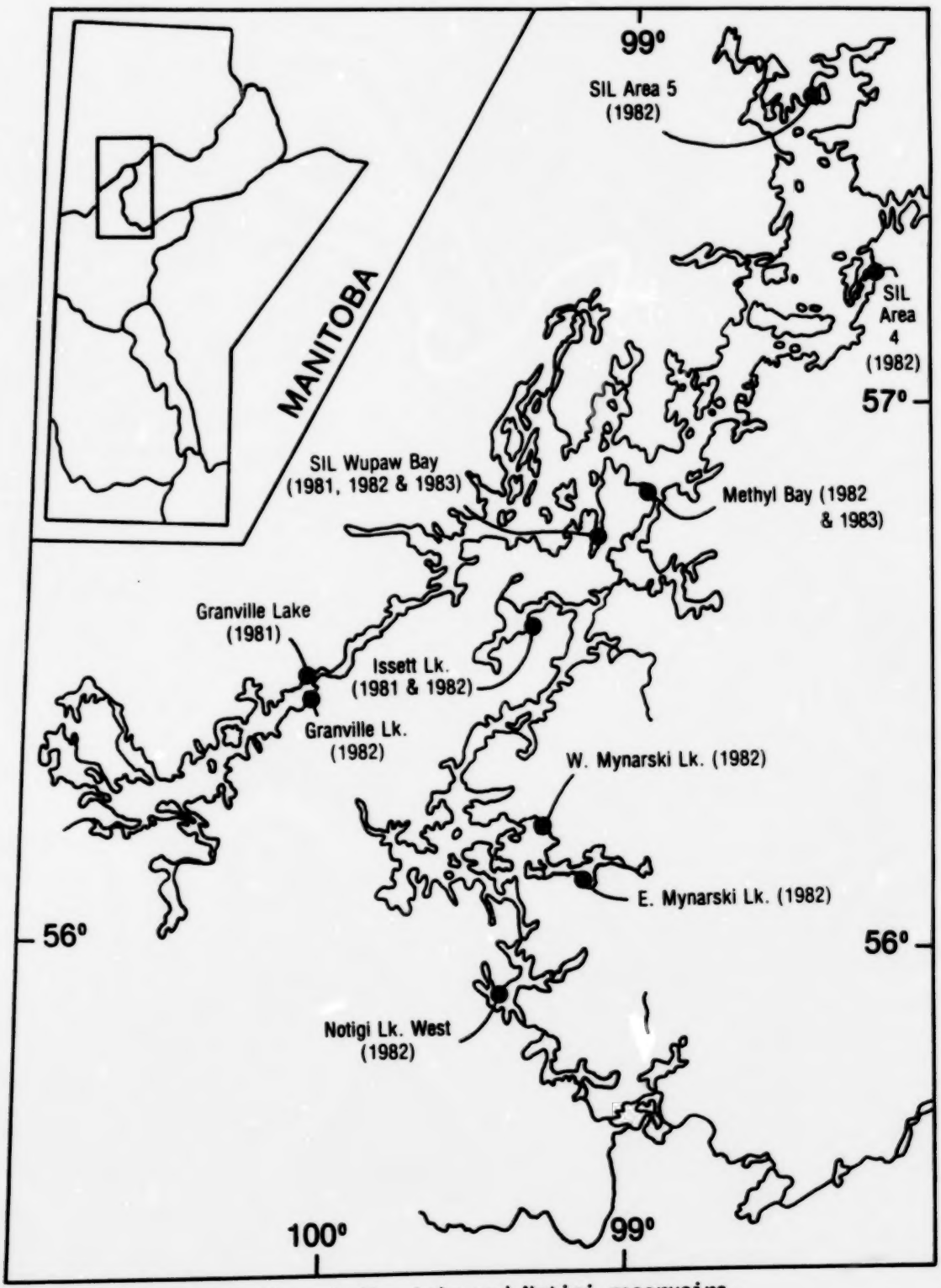
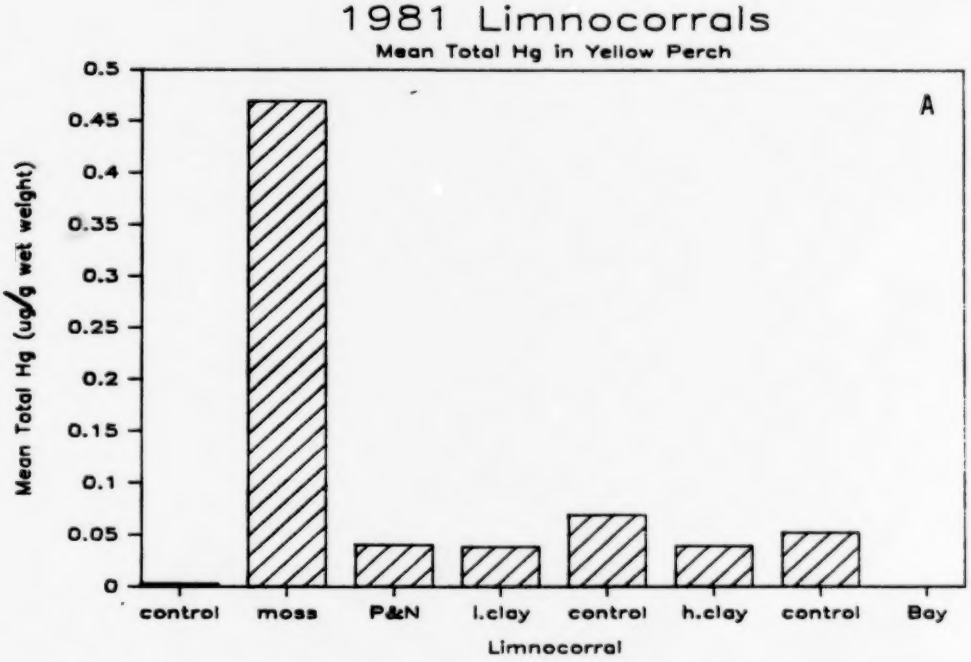


Fig. 1.1. Map of Southern Indian Lake and Notigi reservoirs.



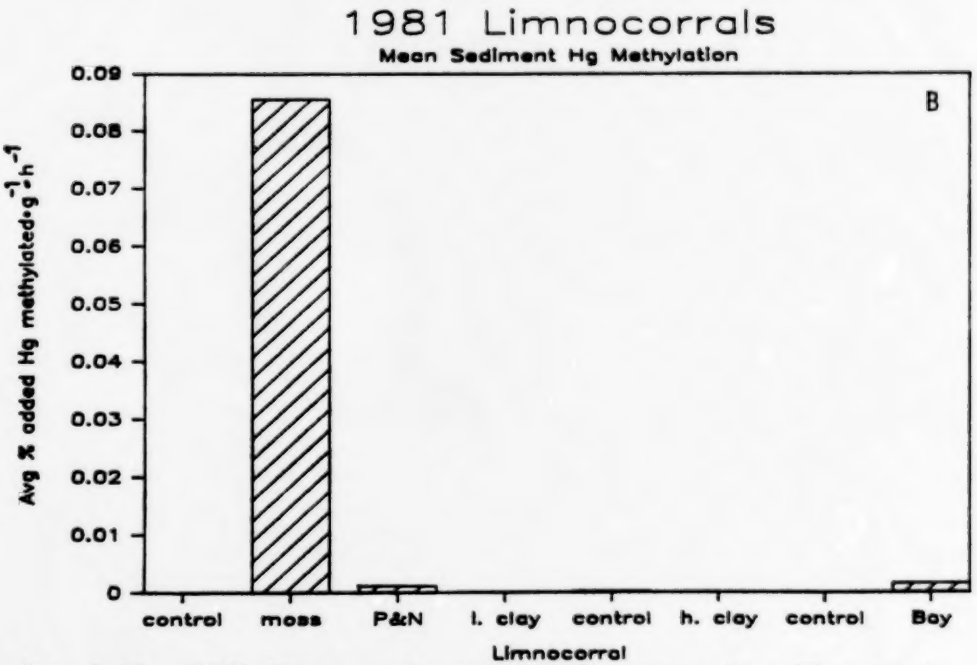
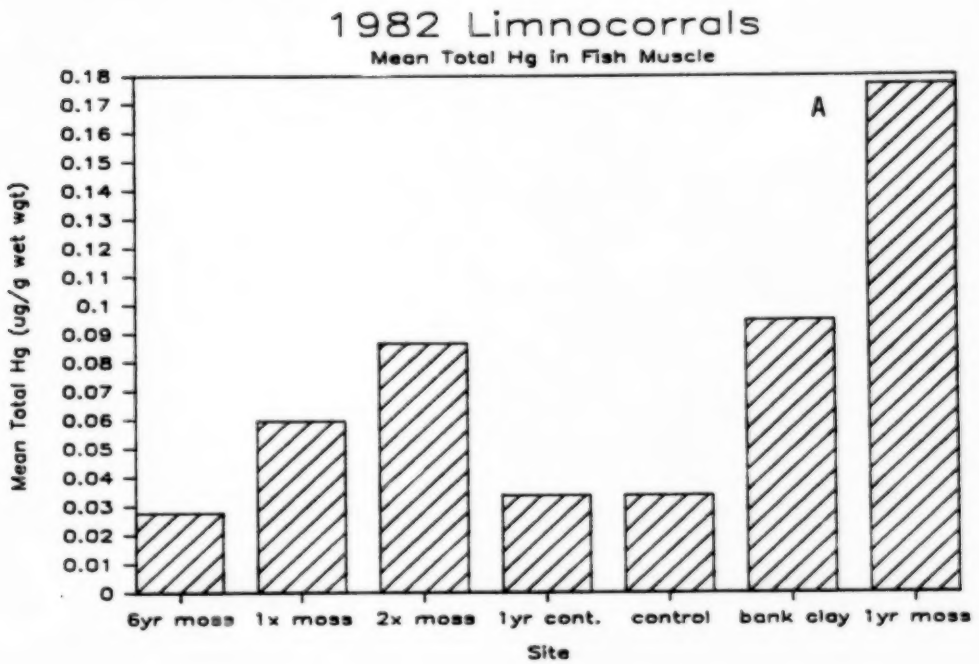


Fig. 1.2A. 1981 limnocorrals-mean total mercury in yellow perch, August 1981 (Hecky et al. 1987).

Fig. 1.2B. 1981 limnocorrals-mean sediment mercury methylation rates. (There are no data available for the samples registering zero on the y-axis.).



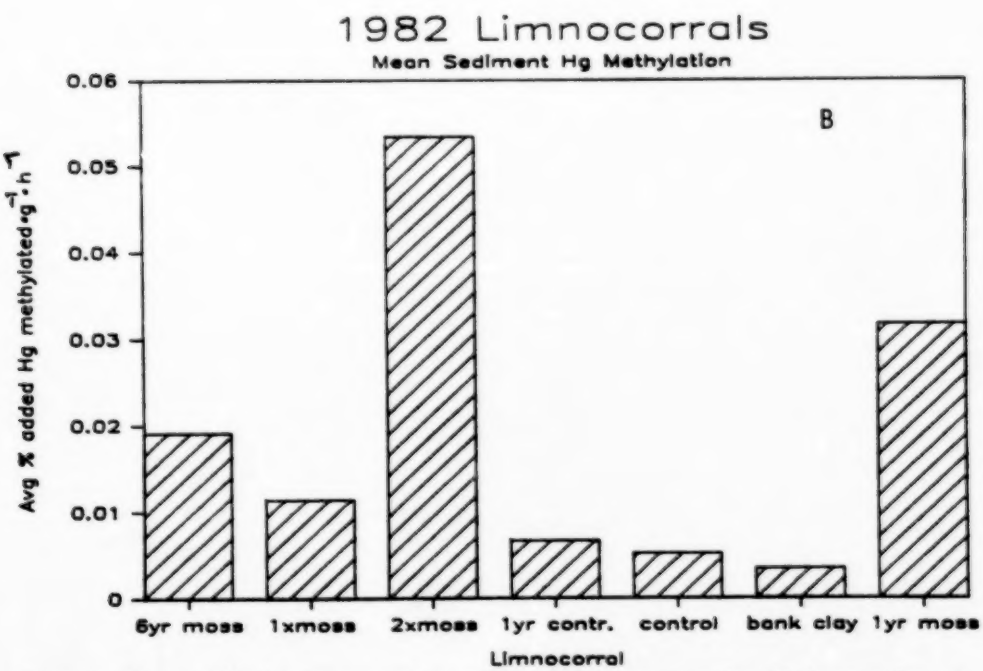
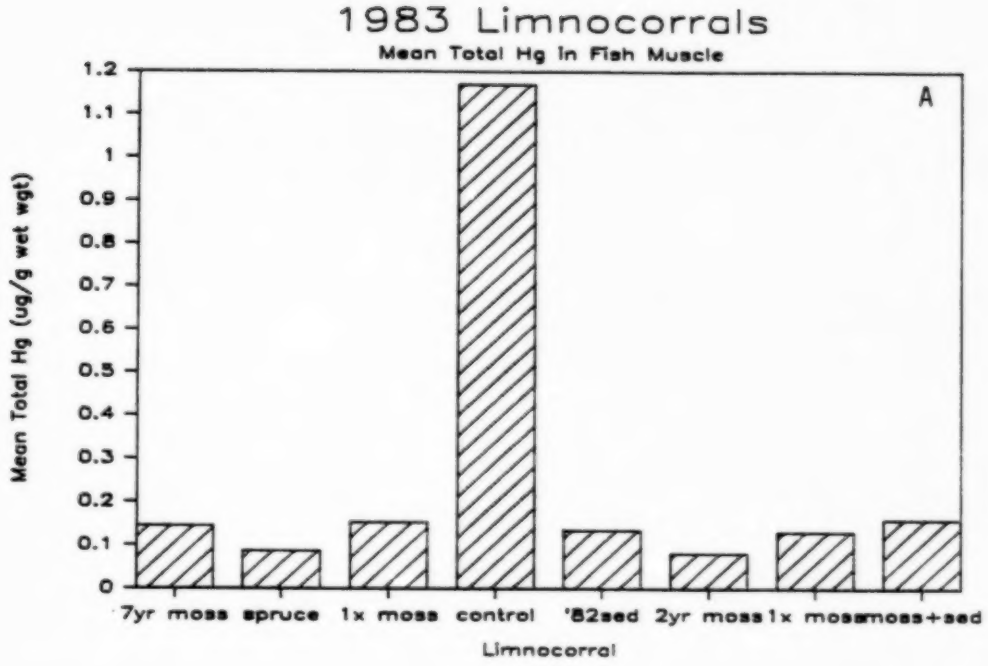


Fig. 1.3A. 1982 limnocorrals-mean total mercury in fish muscle at the end of the experiment (Hecky et al. 1987). There are no data available for the samples registering zero on the y-axis.

Fig. 1.3B. 1982 limnocorrals-mean sediment mercury methylation rates.



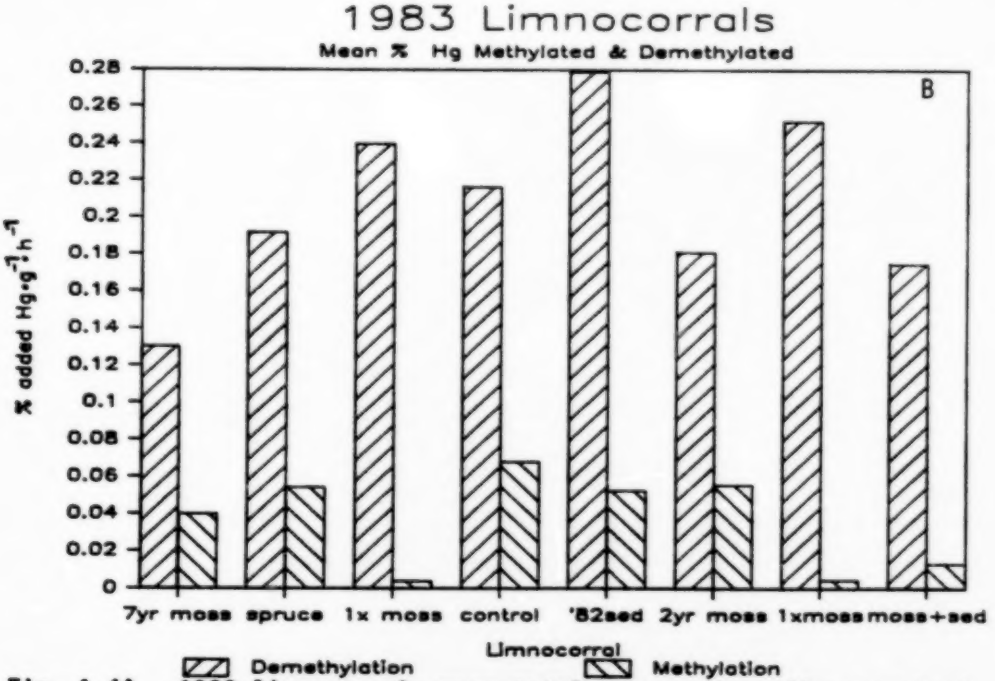
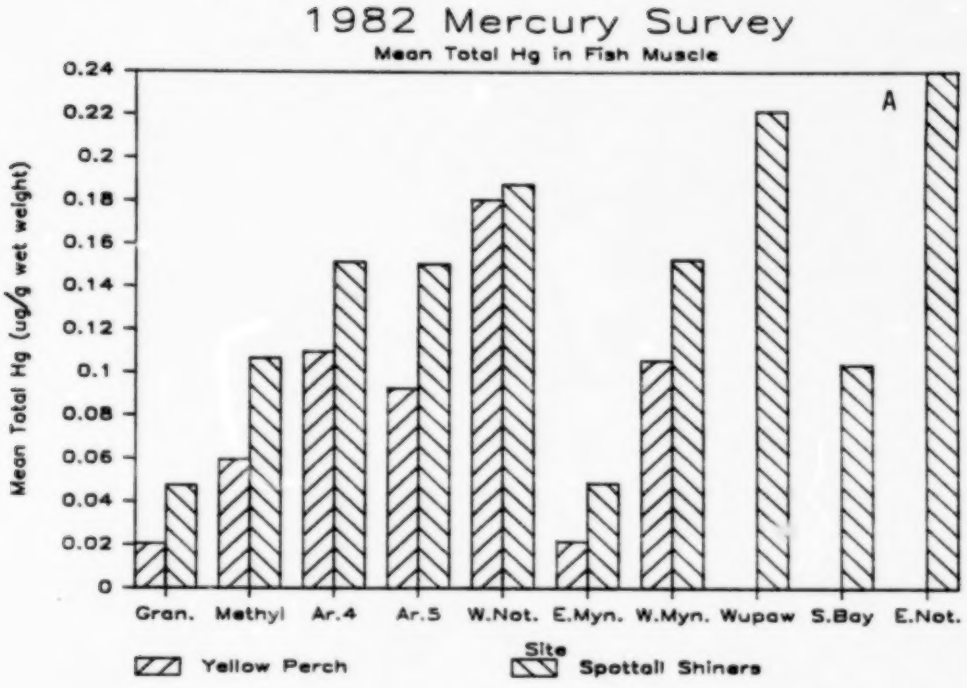


Fig. 1.4A. 1983 limnocorrals-mean total mercury in yellow perch at the end of the experiment (Hecky et al. 1987).

Fig. 1.4B. 1983 limnocorrals-mean sediment mercury methylation and demethylation rates.



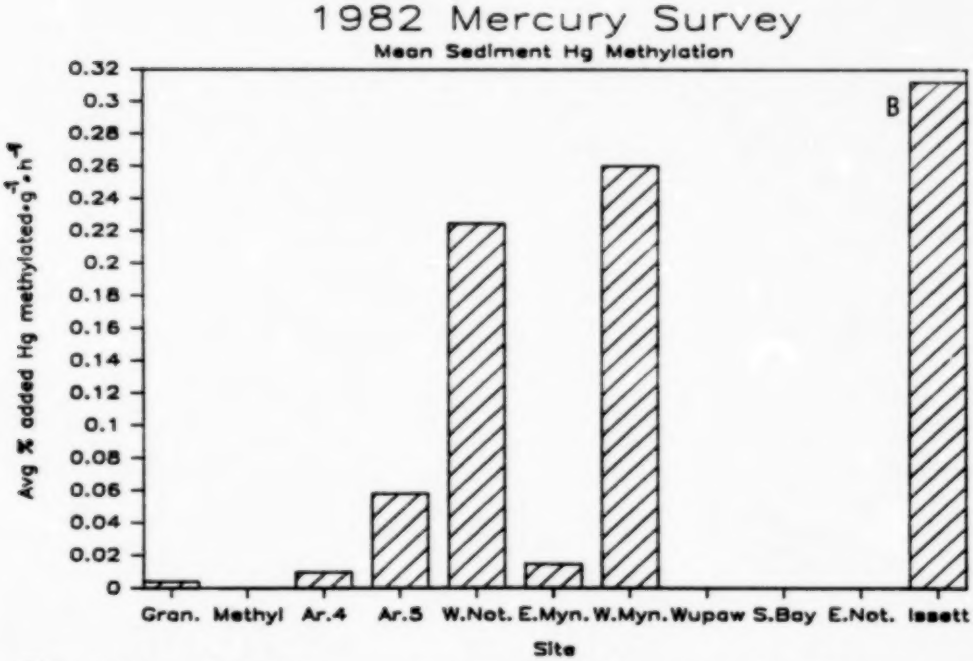


Fig. 2.1A. 1982 mercury survey-mean total mercury in fish muscle (Bodaly et al. 1987).

Fig. 2.1B. 1982 mercury sruvey-sediment mercury methylat rates. West Notigi is given at one-tenth its actual value.

APPENDIX: SEDIMENT CHEMISTRY

Table A1. Chemistry of sediment used for limnocorral methylation studies, 1982. C-carbon and N-nitrogen are expressed as $mg \cdot g^{-1}$ dry weight; R-respiration as $\mu M\ O_2 m^{-3} \cdot h^{-1}$.

Limnocorral	July 8			July 21			August 3			August 23		
	C	N	R	C	N	R	C	N	R	C	N	R
6 yr moss	26	2.8	787	47	4.8	1097	136	9.5	1838	93	7.5	914
1x moss	70	10.1	826	43		986	96	9.1	1456	85	6.7	686
2x moss	19	2.2	1079	53		2058	145	8.3	2669	96	6.1	1644
1 yr cont.	48	7.4				1222	105	8.3	447	63	5.3	810
new cont.	51	4.9	1303	35	5.5	670	119	8.6	875	84	7.9	
bank clay	42	5.5	791			821	57	5.6	385	44	3.8	
1 year moss	51	5.4	2462			1129	99	6.1	490	60	6.0	

Limnocorral	Mean Values C	N	R
6 yr moss	76	6.2	1159
1x moss	74	8.6	989
2x moss	78	5.5	1863
1 yr control	72	7.0	826
new control	72	6.7	949
bank clay	48	5.0	666
1 yr moss	70	5.8	1360

Table A2. pH and rates of carbon dioxide production per gram dry weight of sediments collected from limnocorrals at SIL during 1983.

Limnocorral	July 10, 1983 $\mu molCO_2 \cdot g^{-1} \cdot h^{-1}$	pH	July 25, 1983 $\mu molCO_2 \cdot g^{-1} \cdot h^{-1}$	pH	August 7, 1983 $\mu molCO_2 \cdot g^{-1} \cdot h^{-1}$	pH
7 yr moss (disturbed)	3.08	6.42	0.35	6.60	<0.10	6.66
spruce	0.25	6.54	0.91	6.08	1.10	6.31
1 x moss	0.74	6.97	0.34	6.81	<0.10	6.88
control	0.32	6.69	0.38	6.81	<0.10	6.70
1982 clay addition	-	7.59	0.43	6.72	<0.10	6.70
2 yr moss	<0.10	7.26	0.81	6.68	<0.10	6.69
1x moss	2.53	7.70	<0.10	6.82	<0.10	6.79
1x moss + lake clay	6.84	7.45	0.28	6.83	<0.10	6.85

Table A3: Chemistry of limnocorral sediment expressed as mg carbon (C) or nitrogen (N) per gram dry weight used in mercury methylation studies, 1983.

Limnocorral	July 10		July 25		August 7		Average	
	C	N	C	N	C	N	C	N
7 yr moss (disturbed)	160	9.7	133	8.2	167	10.5	153	9.5
spruce	985	7.6	132	9.8	102	8.5	406	8.6
1 x moss	49	3.0	47	3.0	52	3.5	49	3.2
control	109	12.4	100	8.8	122	9.3	110	10.2
1982 clay addition	74	4.7	72	5.6	72	5.9	73	5.4
2 yr moss	116	7.5	105	7.2	85	7.1	102	7.3
1x moss	49	3.5	53	4.1	63	4.2	54	3.9
1x moss + lake clay	64	4.5	68	4.6	51	3.3	61	4.1

Table A4: Chemistry of sediment used for methylation studies in Methyl Bay, SIL 1982. C-carbon and N-nitrogen expressed as mg·g dry sediment^{-1}; R-respiration as $\mu M\ O_2 \cdot m^{-3} \cdot h^{-1}$.

Site	July 6 C	July 6 N	July 6 R	July 19 R	July 19 R	August 16 C	August 16 N	August 16 R
clay			1170	1027	1405	25	2.7	1433
flooded	57.1	7.5	775	1414	1478	54	5.1	1598
flooded			475	517	1883	90	4.2	1498
clay	44.6	6.3	773	512				
clay				1233	3159	14	1.7	3635

Site	Average Values C	N	R
clay	25	2.7	1259
flooded	56	6.3	1316
flooded	90	4.2	1093
clay	45	6.3	643
clay	14	1.7	2676

Table A5: Chemistry of mercury survey methylation samples, August 17, 1982.

Site	C ($mg \cdot g^{-1}$)	N ($mg \cdot g^{-1}$)	Respiration ($\mu M\ O_2 \cdot m^{-3} \cdot h^{-1}$)
Mynarski E.	80	6.4	1422
Mynarski W.	197	18.1	3084
Notigi W.	73	5.8	2389
Area 5	114	11.8	1429
Area 4	75	3.5	1227
Granville	12	1.8	7855
Isset	98	9.4	

Table A6: Chemistry and rate of carbon dioxide production of sediments (expressed $\mu mol \cdot C \cdot g$ dry weight$^{-1} \cdot h^{-1}$) collected from Methyl Bay, SIL during 1983. C-carbon and N-nitrogen expressed as $mg \cdot g \cdot$dry sediment^{-1}.

	July 19, 1983				August 1, 1983			
Station	CO_2 Prod'n	pH	C	N	C Prod'n	pH	C	N
clay	0.58	6.5	32	3.1	<0.10	6.8	29	2.3
flooded	2.78	6.7	135	5.8	1.39	6.5	85	5.9
flooded	0.11	6.9	99	5.5	<0.10	6.7	178	6.7